consistently associated with measures of function and disability. Most closely associated with function measures were: pain affecting sleep, for WOMAC function; pain catastrophizing, for Late Life basic function, chair stand time, and disability; and pain after 20 m walk, for Late Life advanced function. These findings suggest that different aspects of the pain experience in knee OA may have unique relationships with function and disability. Ultimately, specific multidisciplinary attention to aspects such as the impact of pain on sleep and pain catastrophizing may be a more meaningful approach for the person with painful knee OA, and potentially have greater impact on function and disability over time.

446
ASSOCIATION OF PATIENT-REPORTED OSTEOARTHRITIS SEVERITY WITH OTHER PATIENT-REPORTED OUTCOMES IN THE EUROPEAN CLINICAL SETTING

A. Sadosky[1], A. Bushmakin[2], J. Cappelleri[2], G. Taylor-Stokes[3], J. Sayers[3], D. Carroll[4], T. Gosden[4], P. Emery[5]. *[1]Pfizer, Inc., New York, NY, USA; [2]Pfizer, Inc., New London, CT, USA; [3]Adelphi Real World, Macclesfield, Cheshire, United Kingdom; [4]Pfizer, Inc., Surrey, United Kingdom; [5]Leeds Univ., Leeds, United Kingdom*

Purpose: Better understanding of patients' perceptions of osteoarthritis (OA) severity can improve disease management. However, in clinical practice, categorizing patients as having mild, moderate, or severe OA is not straightforward.
Purpose: To evaluate the association of patient-reported OA severity with other patient-reported outcomes in a European population using the Adelphi Disease Specific Programme for OA (OA DSP VII, 2008).
Methods: OA severity was rated by patients based on the question "How bad would you say your arthritis is now?" with potential responses of "mild," "moderate," and "severe." Other patient-reported outcomes included; a pain visual analogue scale (0 = no pain, 100 = worst possible pain); questions on activities of daily living (1 = no difficulty, 2 = some difficulty, 3 = much difficulty, 4 = unable to do); and the EuroQol health status instrument (EQ-5D). Data on productivity were captured using the Work Productivity and Activity Impairment scale (WPAI). Associations between OA severity and other outcomes were evaluated using descriptive and regression analyses.
Results: Data were available from 1739 patients (63.1% female, mean age 64 years) from France, Germany, Italy, Spain, and the United Kingdom. OA severity was rated as mild, moderate and severe by 24.5%, 56.4%, and 19.2% of the patients, respectively. There was an overall association between patient-rated OA severity and age ($p < 0.05$). Greater patient-reported OA severity was associated with higher pain scores; 28.3, 49.9, and 69.2 for mild, moderate and severe OA, respectively ($p < 0.05$ for pairwise comparisons). As patient-reported OA severity increased, so did functional impairment as indicated by greater difficulty in performing 9 basic and 3 instrumental activities of daily living. After adjustment for gender, the difficulty score was greater than 1 at each OA severity level for every item of functional ability (lower bounds of the 95% confidence intervals were greater than 1); the greatest difficulty was observed among patients who reported severe OA. Pairwise comparisons between OA severity levels showed that differences in functional abilities were statistically significant ($p < 0.05$). Decreases in patient-reported health status with increasing OA severity were also observed on the EQ-5D health state VAS, and the health state index; 0.77, 0.62, and 0.30 for mild, moderate, and severe OA, respectively ($p < 0.0001$ for all pairwise comparisons). Among the 425 patients who were employed, the percent of OA-related impairment of work and activity increased at greater OA severity levels after adjusting for age and gender; higher costs resulting from lost productivity were also observed with increasing OA severity ($p < 0.0001$ for pairwise comparisons).
Conclusions: Patient-reported OA severity was associated with other patient-reported outcomes, including productivity, and these outcomes were most impacted in patients with severe OA. This method provides an accurate and tangible assessment of patient's perceptions of their disease, and may be of benefit in the clinical setting when choosing treatment options for management of OA.

447
PAIN MEDICATION TREATMENT AND JOINT REPLACEMENT SURGERY IN PATIENTS WITH OSTEOARTHRITIS

V. Goldberg[1], C. Datto[2], P. Buzinec[3], R. Morlock[3], H. Yang[2]. *[1]Case Western Reserve Univ., Cleveland, OH, USA; [2]AstraZeneca, Wilmington, DE, USA; [3]Innovus, Eden Prairie, MN, USA*

Purpose: Osteoarthritis (OA) is characterized by joint pain, stiffness, and inflammation and can be associated with a significant number of comorbid conditions. In this retrospective longitudinal study, we estimated the rate of hip and knee replacement surgery in patients with OA and characterized joint replacement, pain medication treatment patterns, and concomitant gastroprotective agent (GPA) use.
Methods: Data were enrollment information and medical and pharmacy claims data from a large, national US health plan. The study population was adult (age ≥18 years) commercial health plan enrollees newly diagnosed with OA during the identification period 1/1/2000–4/30/2009. Patients had ≥2 medical claims with primary OA diagnoses (ICD-9-CM 715.xx, 721.0, 721.2, 721.3, 721.90) ≥30 days apart in the identification period; the index date was the date of the first OA diagnosis. Patients had 1 year of pre-index continuous enrollment with no chronic use of NSAIDs, COX-2 inhibitors (coxibs), or oral opioids, and ≥1 year of post-index enrollment. Outcomes included hip and knee replacement surgery and pain medication use. Demographics, comorbidities, and Charlson Comorbidity Index (CCI) score were measured. Data were analyzed descriptively.
Results: Of 193,829 newly diagnosed patients with OA, 57% were female and 43% were male. Patients whose first observed chronic pain medication use (index pain medications) were strong opioids appeared to have a higher mean CCI score, 1.16, compared with those with NSAIDs, coxibs, and weak opioid index pain medications (0.53–0.83). Patients in the youngest (18–44) and oldest (75+) age groups appeared to be more likely to have an opioid (35.7% and 34.9% respectively) as their index pain medication compared with patients in the middle age groups (45–54 yrs: 24.5%, 55–64 yrs: 19.9%, 65–74 yrs: 24.0%). The incident rates of hip or knee replacement per person-year appeared to be higher for those patients with opioids as their index pain medications (0.0663, weak opioids; 0.0801, strong opioids; 0.0464, NSAIDS; 0.0548, coxibs). The mean time from index pain medication to ANY joint replacement appeared shorter for patients whose index pain medications were opioids (106 days, weak opioids; 28 days, strong opioids; 320 days, nonselective NSAIDs; 479 days, coxibs). Only 18% of patients prescribed NSAIDs were co-prescribed a GPA.
Conclusions: The data from this study indicate specific patterns of relationships between the index diagnosis of OA, joint replacement, pain medication treatment, age, and concomitant GPA use. These data may be important in refining contemporary treatment guidelines, educating physicians in appropriate care paths, and may warrant exploration of potential unmet needs in the management of chronic pain in OA.

Proteomics & Metabolomics

448
PROTEOMICS APPROACH FOR THE SEARCH OF BIOMARKERS IN SERUM AND CARTILAGE OF PATIENTS WITH OSTEOARTHRITIS

P. Fernández-Puente[1], L. Lourido[1], J. Mateos[1], V. Calamia[1], C. Fernández-Costa[1], B. Rocha[1], N. Oreiro[2], C. Fernández-López[2], C. Ruiz-Romero[1], F.J. Blanco[1]. *[1]Osteoarticular and Aging Res. Lab., Proteomics Unit-Associated Node to ProteoRed-ISCIII, INIBIC-CHUAC, A Coruña, Spain; [2]INIBIC-CHUAC. Rheumatology Div., A Coruña, Spain*

Purpose: Osteoarthritis (OA) is a degenerative joint disease that is characterized by cartilage destruction and bone changes, occasionally accompanied by synovial inflammation. A major objective for OA research is the conceptualization and development of early diagnostic strategies. The discovery of protein biomarker panels for early diagnosis, therapeutic purposes and management in several diseases is an area of interest in medicine. In the present work we have quantitatively screened differential proteins in sera and cartilage from patients suffering OA at diverse stages. To attain this objective, a quantitative proteomics approach has been followed, which is based on peptide differential

labelling with iTRAQ reagents and subsequent multidimensional LC-MS/MS analysis.
Methods: 150 serum samples were obtained from OA patients at different stages of the disease (grade II, grade IV and control donors) and were grouped in pools to reduce interindividual variability. The top twenty most abundant proteins in crude serum fluids were removed by affinity chromatography using the immunodepletion column ProteoPrep® 20. Cartilage samples were obtained from OA patients undergoing joint replacement and normal donors without history of joint disease. They were extracted with Urea 6M, 2% SDS using a mixer Mill MM 200 (Retsch). Proteins were quantified, digested with trypsin and differentially labelled with isobaric tags using iTRAQ. The peptide mixture was separated by two dimensional LC coupled to MALDI-TOF/TOF mass spectrometry. Identification and relative quantification of the proteins were performed using ProteinPilot 3.0 software.
Results: Protein profiles between the OA patients (early or late) and healthy controls were compared, leading to the identification of 349 proteins. 28 proteins that were altered in OA with statistical significance and more than 1 peptide: 18 of them were increased (OA:Control >1.3, $p < 0.05$) and 10 decreased (OA:Control <0.7, $p < 0.05$). 263 proteins were identified in cartilage samples and 59 proteins were quantified with a significative p-value in OA patients compared to control. The quantified proteins of cartilage and serum are involved mainly in biological functions as extracellular organization, skeletal system development, cell adhesion, apoptosis, defence and inflammatory response. In both cases it was found that the majority of proteins belonging to the extracellular matrix region. Identification and quantification of some of these proteins both in serum and cartilage from OA patients indicate their potential biomarker value for the pathology. We detected in both samples the alteration of proteins such as cartilage oligomeric matrix protein, lumican, complement factor D or thrombospondin-1, which were found to be increased in OA, whereas the cytoplasmic actin was decreased. Finally, a number of other proteins were identified as altered either in cartilage (thus increasing our knowledge of OA pathogenesis) or in serum (which have also potential biomarker value).
Conclusions: In summary, we have identified for the first time a panel of novel OA protein biomarkers present in serum and cartilage from human patients by a proteomic approach. The specificity and selectivity of these candidates should be verified in order to develop new molecular diagnosis or prognosis tests for OA.

449
DIFFERENCE GEL ELECTROPHORESIS ANALYSIS OF CARTILAGE DEGRADATION IN A MOUSE MODEL OF OSTEOARTHRITIS IDENTIFIES CHANGES IN KEY MOLECULAR PROCESSES

M.D. Gardiner[1], L. Gatto[2], H. Nagase[1]. [1]*Imperial Coll. London, London, United Kingdom;* [2]*Univ. of Cambridge, Cambridge, United Kingdom*

Purpose: The protein profile of cartilage during the initiation and progression of osteoarthritis (OA) is likely to reflect the key early events in the disease pathogenesis. Studying OA progression using human cartilage is challenging owing to the difficulty in acquiring tissue. The surgically induced destabilisation of the medial meniscus (DMM) mouse model provides genetically homogenous cartilage at any disease stage. Proteomic comparison of diseased and control cartilage identifies protein changes occurring in early OA.
Methods: The DMM mouse model was used to study the changes in protein profile of mouse knee articular cartilage during OA. A new microdissection technique enabled targeted collection of pure mouse articular cartilage from the medial tibial plateau. Three pooled biological samples extracted from DMM and control knee joints at 2, 4 and 8 weeks were compared using difference gel electrophoresis (DIGE). Differentially expressed spots were identified by pair wise comparison of normalised spot intensities from control and OA gels. Protein identification from gel spots was performed by tandem mass spectrometry. Histological sections were used to assess cartilage degradation. Immunohistochemistry and real-time RT-PCR were used to further investigate some of the protein changes.
Results: There were no statistically significant differences between DMM and control cartilage at 2 and 4 weeks. Thirty-nine spots out of 1584 (2.5%) were statistically significantly altered in abundance at 8 weeks following DMM surgery. Thirteen distinct proteins from 26 spots were increased in DMM cartilage and 5 distinct proteins from 13 spots were decreased. Small leucine-rich repeat proteins, including biglycan, and fibromodulin, were significantly increased in both protein abundance and gene expression. Gelsolin, a regulator of the actin cytoskeleton, protein disulfide isomerase A3, fibrinogen beta chain and transitional endoplasmic reticulum ATPase were also increased. A number of proteins involved in energy production had an altered abundance; pyruvate kinase, β-enolase and aconitate hydratase were decreased in abundance, glycogen phosphorylase was increased.
Conclusions: Application of a new microdissection technique enabled analysis of the protein profile of pure mouse articular cartilage during OA progression. This technique can be used to isolate and analyse other joint tissues. The changes identified in OA cartilage at eight weeks were consistent with alterations in energy production, the cytoskeleton and cell stress. In addition, the greatly increased abundance of SLRPs adds weight to the importance of these molecules in maintaining cartilage homeostasis. Their roles extend beyond structural interactions to encompass important signalling functions.

450
PHARMACOPROTEOMIC STUDY OF THE EFFECT OF THREE DIFFERENT CHONDROITIN SULFATE COMPOUNDS ON CHONDROCYTE INTRACELLULAR AND EXTRACELLULAR PROTEOMES

V. Calamia[1], P. Fernández-Puente[1], B. Rocha[1], J. Mateos[1], L. Lourido[1], E. Montell[2], J. Vergés[2], C. Ruiz-Romero[1], F.J. Blanco[1]. [1]*Osteoarticular and Aging Res. Lab., Proteomics Unit-Associated Node to ProteoRed-ISCIII, INIBIC-CHUAC, A Coruña, Spain;* [2]*Med. Dept., Bioiberica Pharma, Barcelona, Spain*

Purpose: One of the most widely used compounds in the management of patients with osteoarthritis (OA) is chondroitin sulfate (CS). The aim of this work is to study drug modes of action, side-effects, and toxicity of 3 different CS compounds using two complementary proteomic approaches.
Methods: Chondrocytes were obtained from 3 OA patients undergoing joint replacement. The study was approved by the local Ethics Committee. Proteins expression analysis was carried out using DIGE (Differential In Gel Electrophoresis) and SILAC (Stable Isotope Labeling with Amino acids in Cell culture) techniques. In both cases OA chondrocytes were treated with 200 μg/mL of each brand of CS for 48 hours. For DIGE analysis, proteins samples from control and treated cells were labeled with Cy3 or Cy5 dyes, mixed by pairs and co-resolved by two-dimensional gel electrophoresis using a pool of all samples labeled with Cy2 as internal standard. Quantitative image analysis was performed using Same Spots software. The spots of interest were analyzed by mass spectrometry (MALDI-MS). Protein identification was carried out by database search (MASCOT). For secretome analysis by SILAC, cell-conditioned media from isotope-labeled (treated) and unlabeled (untrated) samples were mixed 1:1, resolved by mono-dimensional gel electrophoresis, and digested with trypsin. Separation and analysis of peptides mixtures was performed by nanoflow liquid chromatography coupled off-line to MALDI-MS. In this case protein identification and quantification was carried out with Protein Pilot software.
Results: We examined around 1500 protein spots that were present in the 6 DIGE gels. We found 46 spots differentially expressed in our conditions: 27 modulated by CS1, 4 by CS2 and 15 by CS3. We didn't observed qualitative changes in the treated cells. We could identify 28 of the altered spots, corresponding to 18 different proteins. CS1 strongly modulated mitochondrial superoxide dismutase (SOD2), a protein previously described by our group as decreased in OA cartilage. The SILAC experiment allowed us to identify 96 different proteins in the CS1 secretome, 95 in CS2 and 104 in CS3. CS1 modulated the expression of 21 proteins: 15 resulted upregulated and 6 downregulated. CS2 modulated 13 proteins: 5 upregulated and 8 downregulated. Finally CS3 modulated 9 proteins: 8 upregulated and only 1 downregulated. Each one of the studied compounds induces a characteristic protein profile in OA chondrocytes cultures. In the case of CS1 more than 60% of the altered proteins are specifically modulated only in this condition. The same occurs with CS3, while most of the proteins modulated by CS2 are in common with CS1 and CS3. Despite the highest number of proteins modulated by CS1, as revealed by DIGE and SILAC experiments, and the increased expression of SOD2, as above mentioned, some of them are catabolic or inflammatory factors like interstitial collagenase (MMP1), stromelysin-1 (MMP3) and pentraxin-related protein (PTX3).